

In re Application of:  
Applicant: Jay M. Short  
Serial No.: 09/663,620  
Filed: September 15, 2000  
Page 13

### REMARKS

Claims 1-62 were pending before this response. By the present communication, new claims 63-115 have been added to define Applicant's invention with greater particularity as shown in attached Exhibit A. The new claim language adds no new matter, being fully supported by the Specification and original claims. Applicant submits that the claim amendments do not narrow the claims in any way within the meaning of Festo Corporation v. Shoketsu Kinzoku Kogyo Kabushiki Co. Ltd., a/k/a SMC Corporation and SMC Pneumatics, Inc. 234 F.3d 558, 51 U.S.P.Q. 2d 1959 (Fed. Cir. 2000). Accordingly, following entry of the amendments in the present response, claims 1-115 are pending and under consideration.

In view of the above amendments and remarks, reconsideration and favorable action on claims 1-113 are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: December 4, 2001



Lisa A. Haile, J.D., Ph.D.  
Registration No. 38,347  
Telephone: (858) 677-1456  
Facsimile: (858) 677-1465

GRAY, CARY, WARE & FREIDENRICH LLP  
4365 Executive Drive, Suite 1100  
San Diego, California 92121-2133

USPTO Customer No. 28213

In re Application of:  
Applicant: Jay M. Short  
Serial No.: 09/663,620  
Filed: September 15, 2000  
Exhibit A: Page 1

**EXHIBIT A**

**A marked up version of the amendments.**

Please add new claims 63-113 as follows:

- 63. (New) A method for obtaining a bioactivity or a biomolecule of interest, comprising:
  - a) screening a library of clones generated from nucleic acids from a mixed population of cells, for a specified bioactivity or biomolecule;
  - b) variegating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and
  - c) screening the variegated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest
- 64. (New) The method of claim 63, wherein the biomolecule is a nucleic acid sequence.
- 65. (New) The method of claim 64, wherein the nucleic acid sequence is a DNA or RNA sequence.

In re Application of:

Applicant: Jay M. Short

Serial No.: 09/663,620

Filed: September 15, 2000

Exhibit A: Page 2

- 66. (New) The method of claim 64, wherein the nucleic acid sequence is screened by contacting the nucleic acids contained in the clone with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of the nucleic acid sequence of interest; and identifying nucleic acid sequences containing a complement to the at least one oligonucleotide probe with an analyzer that detects a detectable signal from the detectable molecule.
- 67. (New) The method of claim 66, wherein the detectable molecule is a chromogenic or a fluorogenic substrate.
- 68. (New) The method of claim 66, wherein the detectable signal is optical fluorescence.
- 69. (New) The method of claim 67, wherein the fluorogenic substrate is umbelliferone or a derivative or analogue thereof, resorufin or a derivative or analogue thereof, fluorescein or a derivative or analogue thereof, or rhodamine or a derivative or analogue thereof.
- 70. (New) The method of claim 66, wherein the detectable molecule is a detectably labeled oligonucleotide having a sequence encoding a polypeptide of interest or a fragment thereof.
- 71. (New) The method of claim 70, wherein the detectably labeled oligonucleotide is labeled with a fluorescent molecule.

In re Application of:

Applicant: Jay M. Short

Serial No.: 09/663,620

Filed: September 15, 2000

Exhibit A: Page 3

- 72. (New) The method of claim 64, wherein the screening is by PCR amplification of a nucleic acid sequence of interest using primers substantially complementary to the sequence of interest or sequences flanking a nucleic acid of interest and having a detectable molecule.
- 73. (New) The method of claim 64, wherein the screening is by hybridization of an oligonucleotide substantially complementary to a nucleic acid sequence of interest and having a detectable molecule.
- 74. (New) The method of claim 63, wherein the bioactivity is provided by a polypeptide.
- 75. (New) The method of claim 63, wherein the bioactivity is an enzymatic activity.
- 76. (New) The method of claim 75, wherein the enzymatic activity is provided by an enzyme selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.
- 77. (New) The method of claim 63 wherein the library is an expression library.
- 78. (New) The method of claim 63, wherein the library contains DNA obtained from an environmental sample.
- 79. (New) The method of claim 78, wherein the environmental sample is selected from ice, water, permafrost, material of volcanic origin, soil and plants.

In re Application of:

Applicant: Jay M. Short

Serial No.: 09/663,620

Filed: September 15, 2000

Exhibit A: Page 4

- 80. (New) The method of claim 63, wherein the library contains DNA obtained from extremophiles.
- 81. (New) The method of claim 80 wherein the extremophiles are thermophiles.
- 82. (New) The method of claim 81, wherein the extremeophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.
- 83. (New) The method of claim 63, wherein the screening comprises contacting a clone with a substrate labeled with a detectable molecule wherein interaction of the substrate with the bioactivity or biomolecule contained in the clone produces a detectable signal.
- 84. (New) The method of claim 83, wherein the substrate is a bioactive substrate.
- 85. (New) The method of claim 83, wherein the bioactive substrate comprises C12FDG.
- 86. (New) The method of claim 83, wherein the screening is by expression of nucleic acid.
- 87. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, GSSM and any combination thereof.

In re Application of:

Applicant: Jay M. Short

Serial No.: 09/663,620

Filed: September 15, 2000

Exhibit A: Page 5

- 88. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by error-prone PCR.
- 89. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by shuffling.
- 90. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by oligonucleotide-directed mutagenesis.
- 91. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by assembly PCR.
- 92. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by sexual PCR mutagenesis.
- 93. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by *in vivo* mutagenesis.
- 94. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by cassette mutagenesis.
- 95. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by recursive ensemble mutagenesis.
- 96. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by exponential ensemble mutagenesis.

In re Application of:

Applicant: Jay M. Short

Serial No.: 09/663,620

Filed: September 15, 2000

Exhibit A: Page 6

- 97. (New) The method of claim 63, wherein the nucleic acid sequence is variegated by site-specific mutagenesis.
- 98. (New) The method of claim 63, comprising screening the clone of (c) for a further specified protein or enzymatic activity, prior to variegating the nucleic acids.
- 99. (New) The method of claim 63, wherein the library is generated in a prokaryotic cell.
- 100. (New) The method of claim 63, wherein the library is generated in a *Streptomyces* sp.
- 101. (New) The method of claim 100, wherein the *Streptomyces* is *Streptomyces venezuelae*.
- 102. (New) The method of claim 99, wherein the prokaryotic cell is gram negative.
- 103. (New) The method of claim 99, wherein the prokaryotic cell is a *Bacillus* sp.
- 104. (New) The method of claim 99, wherein the prokaryotic cell is a *Pseudomonas* sp.
- 105. (New) The method of claim 63, wherein the library is screened by contacting or encapsulating a clone of the library with bioactive substrate, wherein a bioactivity or biomolecule produced by the clone is detectable by a difference in the substrate prior to contacting with the clone as compared to after contacting.
- 106. (New) The method of claim 63, wherein the library is normalized before screening the library.

In re Application of:

Applicant: Jay M. Short

Serial No.: 09/663,620

Filed: September 15, 2000

Exhibit A: Page 7

-- 107. (New) The method of claim 63, wherein the bioactivity or biomolecule is a gene cluster or fragment thereof.

-- 108. (New) The method of claim 63, wherein the bioactivity or biomolecule is a polypeptide in a metabolic pathway.

-- 109. (New) A method for identifying a bioactivity or a biomolecule of interest, comprising:

- a) screening a library for a specified bioactivity or biomolecule wherein the library is generated from pooling individual gene libraries generated from the nucleic acids obtained from each of a plurality of isolates;
- b) variegating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and
- c) screening the variegated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

-- 110. (New) A method of identifying a bioactivity or biomolecule of interest, comprising:

- a) screening a library of clones generated from nucleic acids from an enriched population of organisms for a specified bioactivity or biomolecule;
- b) variegating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and
- c) screening the variegated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.



In re Application of:

Applicant: Jay M. Short

Serial No.: 09/663,620

Filed: September 15, 2000

Exhibit A: Page 8

-- 111. (New) A method for identifying a bioactivity or a biomolecule of interest, comprising:

- a) incubating nucleic acids from a mixed population of organisms with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and such time to allow interaction of complementary sequences;
- b) identifying nucleic acid sequences having a complement to the oligonucleotide probe using an analyzer that detects the detectable molecule;
- c) generating a library from the identified nucleic acid sequences;
- d) screening the library for a specified bioactivity or biomolecule;
- e) variegating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and
- f) screening the variegated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

-- 112. (New) A method for identifying a bioactivity or a biomolecule of interest, comprising:

- a) co-encapsulating in a microenvironment nucleic acids obtained from a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and for such time as to allow interaction of complementary sequences;
- b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable molecule;
- c) generating a library from the separated encapsulated nucleic acids;
- d) screening the library for a specified bioactivity or biomolecule;

In re Application of:

Applicant: Jay M. Short

Serial No.: 09/663,620

Filed: September 15, 2000

Exhibit A: Page 9

- e) variegating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and
- f) screening the variegated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

113. (New) A method for identifying a bioactivity or a biomolecule of interest, comprising:

- a) co-encapsulating in a microenvironment nucleic acids obtained from an isolate of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;
- b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;
- c) generating a library from the separated encapsulated nucleic acids;
- d) screening the library for a specified bioactivity or biomolecule;
- e) variegating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and
- f) screening the variegated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

In re Application of:

Applicant: Jay M. Short

Serial No.: 09/663,620

Filed: September 15, 2000

Exhibit A: Page 10

114. (New) A method for obtaining a bioactivity or a biomolecule of interest, comprising:

- a) co-encapsulating in a microenvironment nucleic acids obtained from one or more isolates of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;
- b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;
- c) generating a library from the separated encapsulated nucleic acids;
- d) screening the library for a specified bioactivity or biomolecule;
- e) variegating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and
- f) screening the the variegated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

In re Application of:

Applicant: Jay M. Short

Serial No.: 09/663,620

Filed: September 15, 2000

Exhibit A: Page 11

115. A method for identifying a bioactivity or a biomolecule of interest, comprising:

- a) co-encapsulating in a microenvironment nucleic acids obtained from a mixture of isolates of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;
- b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;
- c) generating a library from the separated encapsulated nucleic acids;
- d) screening the library for a specified bioactivity or biomolecule;
- e) variegating the a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and
- f) screening the variegated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.